

## 453

## HIP BONE MARROW LESIONS IN ASYMPTOMATIC AND OSTEOARTHRITIC ADULTS: PREVALENCE, RISK FACTORS AND SIGNIFICANCE

L. Dawson<sup>†</sup>, Y. Wang<sup>†</sup>, A.E. Wluka<sup>†</sup>, K. Bennell<sup>‡</sup>, F.M. Cicuttini<sup>†</sup>, <sup>†</sup>Monash Univ., Melbourne, Australia; <sup>‡</sup>Univ. of Melbourne, Melbourne, Australia

**Purpose:** Bone marrow lesions (BMLs) at the knee have an important role in the pathogenesis of osteoarthritis (OA), being associated with increased pain, accelerated cartilage loss, and increased risk of total knee replacement. However, data is limited for the role of BMLs at the hip. Therefore, this study aimed to determine the prevalence and associations of BMLs at the hip in an asymptomatic and an osteoarthritic population.

**Methods:** 142 asymptomatic and 19 participants with hip OA were recruited from existing cohorts. Hip magnetic resonance imaging was performed and used to assess femoral head BMLs, cartilage volume and bone area.

**Results:** The demographic characteristics of the asymptomatic versus the OA population were as follows: age 66.8 ± 7.4 vs. 59.5 ± 7.6 years ( $p < 0.001$ ), female 55.6% vs. 57.9% ( $p = 0.85$ ), body mass index 27.6 ± 4.8 vs. 27.2 ± 4.8 kg/m<sup>2</sup> ( $p = 0.73$ ). The prevalence of BMLs was 17.6% in the asymptomatic population and 63.2% in the OA population ( $p < 0.001$ ). BMLs were strongly associated with OA after adjusting for age, gender and body mass index (odds ratio 5.32, 95% CI 1.78, 15.9,  $p = 0.003$ ). BMLs were associated with lower femoral head cartilage volume in the whole population (regression coefficient -245.7 mm<sup>3</sup>, 95% CI -455.5, -36.0,  $p = 0.02$ ). In the OA population, BMLs were also associated with lower femoral head cartilage volume (regression coefficient -426.6 mm<sup>3</sup>, 95% CI -855.2, 2.14,  $p = 0.05$ ) after adjusting for age, gender, body mass index, femoral head bone area and hip OA (for analysis of the total population).

**Conclusion:** Femoral head BMLs are common in those with OA, but are also present in asymptomatic individuals with no clinical hip OA. They are associated with reduced hip cartilage volume. These findings suggest that BMLs at the hip may provide a novel target for the treatment and prevention of hip OA.

## 454

HYDROGEN SULFIDE DONORS ALLEVIATE IL-1 $\beta$  INDUCED INFLAMMATION-LIKE EFFECTS IN HUMAN ARTICULAR OSTEOARTHRITIC CHONDROCYTES

E.F. Burguera<sup>†,‡</sup>, A. Vela-Anero<sup>‡,§</sup>, R. Meijide-Failde<sup>§</sup>, F.J. Blanco<sup>†,‡</sup>, <sup>†</sup>INIBIC-Hosp. A Coruña, Rheumatology Div., A Coruña, Spain; <sup>‡</sup>Tissue Engineering and Cellular Therapy Group (CBTTC-CHUAC), CIBER-BBN/ISCIII, A Coruña, Spain; <sup>§</sup>Cell Therapy and Regenerative Med. Group, Dept. of Med., Univ. of A Coruña, A Coruña, Spain

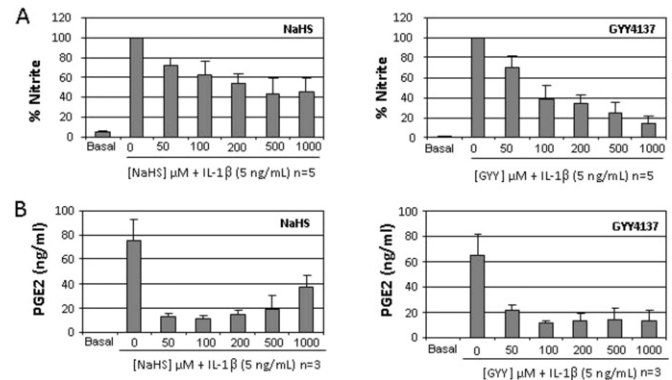
**Purpose:** Hydrogen sulfide, H<sub>2</sub>S, has been recently recognized as an important signaling molecule. Once exclusively thought of as a toxic gas, it is now being considered as the third gas transmitter together with NO and CO. H<sub>2</sub>S has been found to be implicated in many inflammatory pathologies and there are studies that suggest that it can act as an anti-inflammatory and anti-oxidant agent. In the present study, we analyzed the influence of two H<sub>2</sub>S donors (NaHS and GYY4137) on several aspects that characterize the inflammatory process in osteoarthritis (OA). Specifically we looked at: 1) Nitric oxide (NO) production and inducible NO synthase (iNOS) levels; 2) Production of reactive oxygen species (ROS) and antioxidant enzyme superoxide dismutase 2 (SOD2); 3) The production of prostaglandin E-2 (PGE-2); and 4) Levels of matrix metalloproteinase 3 (MMP3).

**Methods:** Primary human chondrocytes were isolated from OA tissue. Cells were stimulated with a pro-inflammatory cytokine (interleukin-1 $\beta$ , IL-1 $\beta$ , 5 ng/ml) and we used different concentrations (ranging from 50  $\mu$ M to 1000  $\mu$ M) of the two H<sub>2</sub>S donors to investigate their ability to ameliorate the effects of IL-1 $\beta$  on the cells. NO production was quantified through the Griess reaction. Protein levels were visualized through immunocytochemistry and quantified with appropriate software; mRNA expression levels were detected with qRT-PCR. ROS levels were quantified with a fluorescence microscope after DCFH or dihydrorhodamine 1,2,3 treatment. PGE-2 levels were measured with a specific EIA.

**Results:** Treatment with IL-1 $\beta$  caused an increase in NO and PGE-2 production, iNOS protein levels, and SOD2 and MMP3 mRNA and protein levels. GYY4137 and NaHS treatment was effective in reducing NO production down to 25% and 50% of the stimulated values (respectively), although none were able to recover the non stimulated

state (Figure 1A). Even though H<sub>2</sub>S might react directly with it, NO reduction was probably the result of a decline in iNOS stimulation, because iNOS protein levels were also reduced by both GYY4137 and NaHS treatment. H<sub>2</sub>S released by GYY4137 and NaHS probably reacts directly with the ROS present in the OA cells and we saw a reduction in both mRNA and protein levels of SOD2 in the stimulated cells. These effects were also accompanied by a reduction in PGE-2 levels: all GYY4137 concentrations led to about a 60% reduction in PGE-2 in the stimulated cells and low NaHS concentrations (50–200  $\mu$ M) to about a 75% reduction (Figure 1B). We also saw reductions in both MMP3 mRNA and protein levels after treatment with the H<sub>2</sub>S donors.

**Conclusions:** Results obtained so far suggest that there might be a therapeutic window for H<sub>2</sub>S donors that show anti-inflammatory and antioxidant properties. This might be of interest in the alleviation of OA-induced inflammation processes and it should be further explored.



**Figure 1.** Effect of different concentrations of H<sub>2</sub>S donors NaHS and GYY4137 on (A) NO and (B) PGE-2 production in IL-1 $\beta$  stimulated human articular osteoarthritic chondrocytes.

## 455

## DOWNREGULATION OF WNT SIGNALING PATHWAY AND MIR335 THROUGH MIR335 IN MSCS FROM OSTEOARTHRITIS PATIENTS

B. Fernandez-Gutierrez, P. Tornero-Esteban, J.R. Lamas, E. Villafuertes, A. Peralta-Sastre, M. Hernandez-Molinero, J.A. Hoyas, UGC de Reumatología, Hosp. Clínico San Carlos, Inst. de Investigación Sanitaria del Hosp. Clínico San Carlos (IdISSC), Madrid, Spain., Madrid, Spain

**Purpose:** Wnt signalling pathway regulates mesenchymal stem cell (MSC) self-renewal and differentiation. In osteoarthritis (OA) the Wnt signalling is altered in MSCs isolated from the bone marrow (BM-MSC) and likely contributing to OA pathogenesis. miRNAs are critical regulators of mesenchymal stem biology and overexpression of specific miRNA (mir335), which in addition is upregulated by the Wnt/ $\beta$ -catenin signalling pathway, has been shown to inhibit osteogenic differentiation. According to these evidences, the aim of this study was to delineate the effect over Wnt genes after mir335 overexpression in BM-MSCs from OA patients.

**Methods:** RNA samples from BM-MSC of three OA patients and three controls were analyzed by quantitative PCR to determine the expression of mir335 and MEST gene (which controls mir335 expression). The effect of mir335 overexpression in Wnt signaling was determined using a GFP gene reporter assay of BM-MSC transduced with a lentiviral vector containing mir335 and GFP in one OA patient. Transduced (GFP+) cells were purified 48h after transduction by fluorescence-activated cell sorting (FACS). Transduced GFP cells as well as non-transduced cells were used as control. Cells were further induced to osteogenic differentiation and studied at 0, 10 and 21 days. Simultaneous expression of 84 Wnt signalling pathway related genes were analyzed by a PCR Array profiling.

**Results:** mir335 expression levels were reduced by about 50% in OA patients compared to expression levels found in controls. As expected, MEST gene was also clearly downregulated. A different behaviour was observed during differentiation to osteoblast lineage. At initial stages (0–10 days) BM-MSC transduced with mir335 showed an up-regulation of 16 Wnt signalling pathway related genes. Interestingly, seven out of the 16 were downregulated in the non-transduced cells. These genes includes the PITX2, SFRP1, SFRP4, WIF1, WNT16, WNT2 and WNT6. Towards the terminal stage of bone forming cells (10–21) days, a marked reduction of gene expression was detected in comparison to control

(non-transduced cells). A set of 53 downregulated genes pertaining to the, canonical, planar, Cell Growth & Proliferation and Cell Migration, were identified. One of them, DAAM1, have been already described as direct targets of mir-335.

**Conclusions:** Our results indicate a lower expression of mir335 in OA-MSCs patients and suggest that the downregulation in Wnt related genes during the initial stages of differentiation that could be partly restored after mir335 overexpression. Therefore, we hypothesize that a diminished expression of mir335 could contribute to the altered function of MSCs in OA.

#### 456

##### LONGITUDINAL RATES OF CHANGE IN SUBCHONDRAL BONE SIZE IN HEALTHY KNEES AND KNEES WITH RADIOGRAPHIC OSTEOARTHRITIS

M. Hudelmaier<sup>†</sup>, W. Wirth<sup>†</sup>, M. Nevitt<sup>‡</sup>, F. Eckstein<sup>†</sup>. for the OAI investigators<sup>†</sup> *Paracelsus Med. Univ., Salzburg, Austria*; <sup>‡</sup> *Univ. of California, OAI Coordinating Ctr., San Francisco, CA, USA*

**Purpose:** Several studies reported that an increase of subchondral bone area (TAB) is associated with features of radiographic knee osteoarthritis (rOA), such as osteophytes, joint space narrowing or subchondral defects. One cross-sectional study reported that TABs were larger in knees with higher KL grades, based on site readings from the OA initiative (OAI). Here we test the hypotheses that knees with rOA show greater rates of longitudinal TAB change than knees with risk factors but not definite radiographic OA (pre-rOA) or healthy knees without.

**Methods:** Coronal FLASHwe MR images of 899 right knees from OAI participants (539 women, 360 men; age  $61.6 \pm 9.5$ ; BMI  $28.9 \pm 4.8$ ) were acquired at baseline and 12 month follow-up (public use datasets 0.E.1 and 1.E.1). Based on central radiographic readings (Boston University), 101 knees were classified asymptomatic healthy controls (bilateral KLG 0, no OA risk factors), 254 were pre-rOA (KLG 0&1, with risk factors), and 544 had definite rOA (KLG 2-4). The TAB of the medial and lateral tibia (MT/LT) and weight-bearing (central) femoral condyles (cMF/cLF) were segmented by experienced readers, by matching numbers of slices processed per plate in scan pairs, but with blinding to acquisition order. The size of the TAB was calculated in 3D. Because an increase in TAB was expected, 1-sided non-paired t-tests were used to compare groups, and significance of change was assessed with 1-sided paired t-tests.

**Results:** TAB changes were less than 1% in all strata (Table 1) and were not significantly different from zero in healthy reference knees (SRM range -0.10 to +0.11). Pre-rOA knees had a significant ( $p < 0.05$ ) TAB increase in cMF (SRM +0.10) but not in other plates (SRM  $\leq 0.05$ ). rOA knees showed significant TAB increases in MT (SRM +0.14), cMF (SRM +0.21) and cLF (SRM +0.15), but not in LT (SRM +0.03). The changes were significantly greater than in rOA than in pre-rOA knees for MT and cLF ( $p < 0.05$ ), and greater than in healthy controls for MT ( $p < 0.05$ ). In MT, the percent increase in TAB was greater in knees with higher KL grades.

**Conclusions:** Knees with definite rOA show small but significant longitudinal increases in TAB, predominantly in the medial compartment. In the medial tibia, the rate of TAB change was greater in rOA than in pre-rOA or healthy knees. The observed relationship between TAB change and rOA status in the medial tibia may be due to rOA predominantly affecting the medial compartment (in general and in the OAI) and because femorotibial loading is known to be greater medially than laterally. The current results suggest that longitudinal change in TABs is a feature of structural progression associated with rOA status.

**Table 1**

Change in the subchondral bone area (TAB [Mean% $\pm$ SEM]) over 12 months in OAI knees

Group	Size	MT	LT	cMF	cLF
Healthy	n=101	-0.1 $\pm$ 1.2%	-0.1 $\pm$ 1.2%	0.2 $\pm$ 1.1%	0.0 $\pm$ 0.9%
Pre-rOA	n=254	0.0 $\pm$ 0.8%	0.1 $\pm$ 0.7%	0.2 $\pm$ 0.6%	0.0 $\pm$ 0.5%
KLG2	n=316	0.1 $\pm$ 0.7%	0.1 $\pm$ 0.7%	0.3 $\pm$ 0.5%	0.2 $\pm$ 0.5%
KLG3	n=167	0.2 $\pm$ 1.1%	0.1 $\pm$ 1.0%	0.5 $\pm$ 0.9%	0.2 $\pm$ 0.8%
KLG4	n=61	0.3 $\pm$ 2.1%	-0.1 $\pm$ 1.9%	0.3 $\pm$ 1.5%	0.3 $\pm$ 1.1%

#### 457

##### PPAR $\alpha$ SIGNALLING REDUCES SUBCHONDRAL BONE THICKENING AND INFLAMMATION, BUT DOES NOT PREVENT CARTILAGE DAMAGE IN STR/ORT MICE

W. Wei<sup>†</sup>, S. Botter<sup>†,‡</sup>, Y. Bastiaansen-Jenniskens<sup>†</sup>, L. Gierman<sup>||,§</sup>, S. Bierma-Zeinstra<sup>†</sup>, J. Verhaar<sup>†</sup>, H. Weinans<sup>†</sup>, A.-M. Zuurmond<sup>§</sup>, S. Clockaerts<sup>†,¶</sup>, G. Van Osch<sup>†</sup>. <sup>†</sup> *Erasmus Univ. Med. Ctr., Rotterdam, The Netherlands*; <sup>‡</sup> *Univ. Hosp. Balgrist, Zurich, Switzerland*; <sup>§</sup> *TNO, Leiden, The Netherlands*; <sup>||</sup> *Leids Univ. Med. Ctr., Leiden, The Netherlands*; <sup>¶</sup> *Univ. of Antwerp, Antwerp, Belgium*

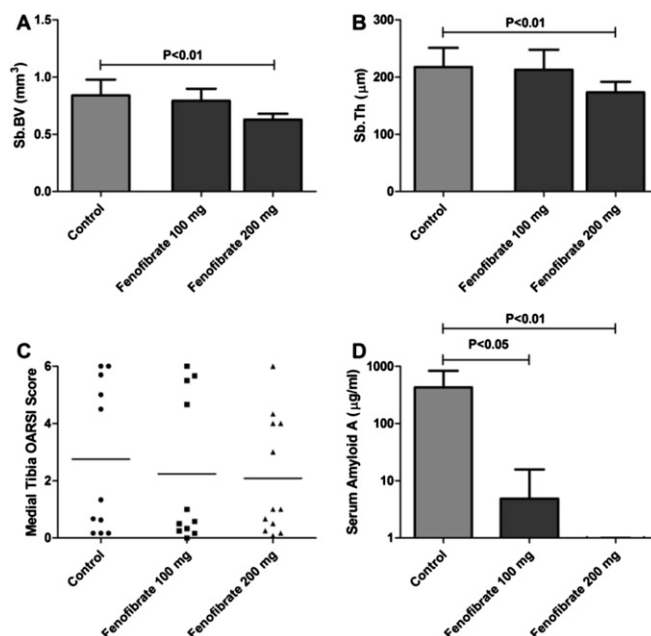
**Purpose:** Subchondral bone changes are an important feature in osteoarthritis (OA). Initial loss of bone volume (BV) followed by sclerosis and thickening of the subchondral bone plate occur during OA development. STR/Ort mice have been suggested to develop OA spontaneously due to reduced endogenous PPAR $\alpha$  signalling, possibly by increasing inflammatory processes in the joint and an altering osteoblast phenotype [Watters 2007, Arthritis Rheum]. To confirm the role of PPAR $\alpha$  in the development of OA, we have treated STR/Ort mice with fenofibrate, a PPAR $\alpha$  agonist, and evaluated inflammation, subchondral changes and OA cartilage damage in this spontaneous OA model.

**Methods:** 8-weeks-old male STR/Ort mice (n=36) were divided into 3 experimental groups (n=12 per group). One group received reference diet (Control) whereas the other 2 groups received reference diet mixed with two different dosages of fenofibrate (100 mg/kg bodyweight (BW)/day or 200 mg/kg BW/day). Mice were euthanized after 16 weeks of treatment at the age of 6 months.  $\mu$ CT was used to quantify subchondral bone volume (Sb.BV) in a pre-defined region of interest and subchondral plate thickness (Sb.Th), in addition to metaphyseal bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp). Level of inflammation was determined by measuring serum amyloid A (SAA). Knee cartilage damage was evaluated by histology using the OARSI scoring method.

**Results:** Mice treated with 200 mg/kg BW/day fenofibrate had a significantly lower amount of subchondral bone, with lower Sb.BV and Sb.Th, as well as a lower amount of metaphyseal bone, with lower BV/TV and Tb.N and elevated Tb.Sp. No significant difference in cartilage damage was found between the groups. Fenofibrate treated mice had significantly lower levels of SAA compared to Control. Although no difference was found in SAA levels between mice with and without cartilage damage, reduced SAA was associated with less Sb.BV, BV/TV and Tb.Th.

**Conclusions:** Systemic inflammation and subchondral bone volume and thickness in the STR/Ort mouse can be reduced by fenofibrate treatment. However, cartilage damage was not influenced. This suggests that PPAR $\alpha$  signalling may influence subchondral bone morphology, but is not correlated with cartilage damage in the STR/Ort mouse.

**Acknowledgment:** financial support of Top Institute Pharma (grant T1-213-1), Anna Fonds and Nuts Ohra



**Figure.** Effect of treatment on (A) subchondral bone volume and (B) plate thickness, (C) cartilage damage and (D) serum amyloid A levels. Indicated dosage is mg/kg bodyweight/day.